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Detection of antisynthetase syndrome in patients with idiopathic interstitial pneumonias

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KEYWORDS
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Summary
Objectives: Antisynthetase syndrome (ASS) is characterized by autoantibodies to aminoacyl-tRNA synthetases (anti-synthetase) and it is frequently associated with interstitial lung disease. The purpose of this study was to elucidate the prevalence and characteristics of the anti-synthetase positive subpopulation among idiopathic interstitial pneumonias (IIPs) and to clarify the importance of screening for these antibodies.
Methods: A retrospective study was performed in 198 consecutive cases with IIPs. Screening for six anti-synthetase antibodies was performed in all cases. Clinical profiles of all cases were compared with reference to the presence of anti-synthetase. High-resolution computed tomography (HRCT) findings of anti-synthetase positive cases were also analyzed.

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Results: 13 cases (6.6%) were positive for anti-synthetase. Anti-EJ was most prevalent, followed by anti-PL-12. Onset ages of anti-synthetase positive cases were younger than those of anti-synthetase negative cases. Extrapulmonary features of ASS were absent in 6 anti-synthetase positive cases (46.2%). Histologically, among 5 UIP with lymphoid follicles and 11 NSIP cases, the prevalence of anti-synthetase positive cases was 8/16 (50%). On HRCT, ground glass opacity and traction bronchiectasis were the major findings in anti-synthetase positive cases, while honeycombing was absent.
Conclusions: Anti-synthetase positive cases were not rare among IIPs. Anti-synthetase should be screened for in IIPs, especially in pathological NSIP or UIP with lymphoid follicles. These patients should be screened for anti-synthetase even if no suggestive extrapulmonary manifestation exists.

Introduction

Interstitial lung disease (ILD) is caused by both known and unknown etiologies, and idiopathic interstitial pneumonias (IIPs) are the most prevalent type of ILD.[1] Connective tissue disease (CTD) affects a wide variety of organs with the incidence of pulmonary involvement ranging from 19 to 67%.[2] A thorough etiological work-up of CTD and its related conditions is essential in clinical practice for ILD. Difficult-to-diagnose cases exist when they have an incomplete form of CTD that cannot be categorized as CTD using a range of specific criteria. These cases are categorized as idiopathic, or sometimes unclassifiable.

The aminoacyl-tRNA synthetases are a family of enzymes, each of which catalyses the formation of aminoacyl-tRNA from a specific amino acid and its cognate tRNA. Autoantibodies to eight of these synthetases (anti-synthetase) have been reported and they are defined as myositis-specific autoantibodies.[3] Clinical features that occur in anti-synthetase positive cases include myositis, ILD, arthritis, mechanic's hand and often Raynaud's phenomenon. Combination of positive anti-synthetase antibody with any of these findings constitutes a distinct syndrome named anti-synthetase syndrome (ASS).[4] The prevalence of anti-synthetase among PM/DM is 30—40% and characteristics of anti-synthetase positive populations have been established in the past, in several myositis-based large cohort studies. In particular, subpopulations of patients with myositis have higher rates of ILD when they have anti-synthetase than those who have not.[5] ILD in ASS may also be indistinguishable from IIPs when patients have minimal or no myositis. ASS comprises a distinct disease entity and demonstrates a generally good response to corticosteroids, though a number of cases show recurrence after withdrawing or reducing doses of corticosteroids.[6,7] Fisher et al. retrospectively assessed 37 patients with IIPs who had some signs or symptoms indicative of ASS, but were not positive for ANA or anti-Jo-1 antibody, and found that 9 (24%) patients were positive for anti-synthetase.[8] In this previous study, anti-synthetase were measured in patients with clinical signs indicative of ASS, and the decision to perform the test depended upon each physician; thus the overall prevalence of anti-synthetase among IIPs was not clarified. The characteristics of patients with anti-synthetase positive IPs are still unknown together with identification of the population which should be examined for these antibodies. This study aimed to investigate the prevalence of an anti-synthetase positive subpopulation among IIPs. Six anti-synthetase antibodies were measured and aspects of the clinical, pathological and radiological features of anti-synthetase positive cases were investigated to determine which patients should be screened for these antibodies.

Methods

Patient recruitment

In this study, idiopathic interstitial pneumonia (IIP) was defined as interstitial pneumonias of unknown cause where a patient did not fulfill classification criteria for any specific CTD or vasculitides, and in whom lung diseases were not potentially caused by drug or occupational-environmental exposures. Screening for CTD was initially performed by experienced pulmonologists, and also by rheumatologists in patients with serological or clinical features suggestive of CTD.[9—16]Provisionally, undifferentiated connective tissue disease (UCTD)[17] were not excluded from the study population. In all patients diagnosed with idiopathic interstitial pneumonias (IIPs) who visited Kyoto University Hospital from July 2007 through April 2009 and Tenri Hospital, the tertiary care center from April 2006 through April 2009, serum samples were consecutively collected and patients were recruited into this study. The study population comprised 198 cases with IIPs, 53 with idiopathic pulmonary fibrosis/usual interstitial pneumonia (IPF/UIP) (30 by histological diagnosis; 23 by clinical diagnosis), 11 with nonspecific interstitial pneumonia (NSIP), 3 with histologically unclassifiable interstitial pneumonia, and 131 with non-UIP without histology. Written informed consent was obtained from the participants, and the study was approved by the Review Board of the Ethical Committee of each Institute.

Data collection

Clinical information was retrospectively obtained from medical records. The data included patient's age at onset, gender, pulmonary or extrapulmonary manifestations including signs or symptoms of CTD, laboratory data results including CTD-specific autoantibodies, blood gas analysis, pulmonary function test results, bronchoalveolar lavage

fluid (BALF) findings, treatment regimen, definitive diagnosis of polymyositis/dermatomyositis (PM/DM) during observation, and survival. Clinical diagnosis of PM/DM was made according to the criteria of Bohan and Peter.[16]

Measurement of anti-synthetase

Investigation of anti-synthetase was performed by an RNA immunoprecipitation procedure (IPP) using sera and HeLa cell extracts as previously described.[7] Briefly, 10 μl of sera was mixed with protein-A sepharose beads and incubated with sonicated extracts of HeLa cells. RNAs were extracted with phenol-chloroform and then resolved in urea-10% polyacrylamide gel, which was finally visualized with silver staining (Bio-Rad Laboratories, Hercules, CA, USA). Each antibody was identified by the mobility and pattern of tRNA bands (Fig. 1). Among the eight antibodies established in previous reports, we investigated the following six antibodies; antibodies to histidyl- (Jo-1), threonyl- (PL-7), alanyl- (PL-12), isoleucyl- (OJ), glycyl- (EJ), and asparaginyl-(KS) tRNA synthetases. The other two antibodies have only been reported in case presentations, and are considered to be rare; thus they were not investigated in this study.[3]

Assessment of high-resolution computed tomography (HRCT) findings

Chest high-resolution computed tomography (HRCT) findings were assessed independently by two radiologists (S. N. and T. K.) who were unaware of the clinical or pathological data. The lung fields in HRCT were divided into upper, middle and lower zones at the level of the carina and inferior pulmonary vein, and findings were assessed separately in the three zones of both lungs. The presence or absence of the following nine findings were reported in each subdivided lung zone: pleural irregularities and/or prominent interlobular septa, reticulation, ground glass opacity, consolidation, subpleural lines, centrilobular nodular opacity, irregular peribronchovascular thickening, traction bronchiectasis and bronchiolectasis, honeycombing.[18–20] Initially two readers evaluated the images and recorded results independently. After the completion of separate assessment, unified consensus results were reached through discussion between the two readers.

Statistical analysis

Data were summarized as a median and an interquartile range. We used the Wilcoxon rank sum test to compare the numerical variables and chi-square test or Fisher's exact test as appropriate to the categorical variables. Interobserver variances in the initial assessment of HRCT findings were evaluated using the kappa coefficient for each finding. Kaplan–Meier survival curves were derived for the study population, and comparisons were made using the log rank test. We used JMP (version 6, Japanese Edition. SAS Institute Inc.) in statistical analysis. A p-value of less than 0.05 was considered significant.

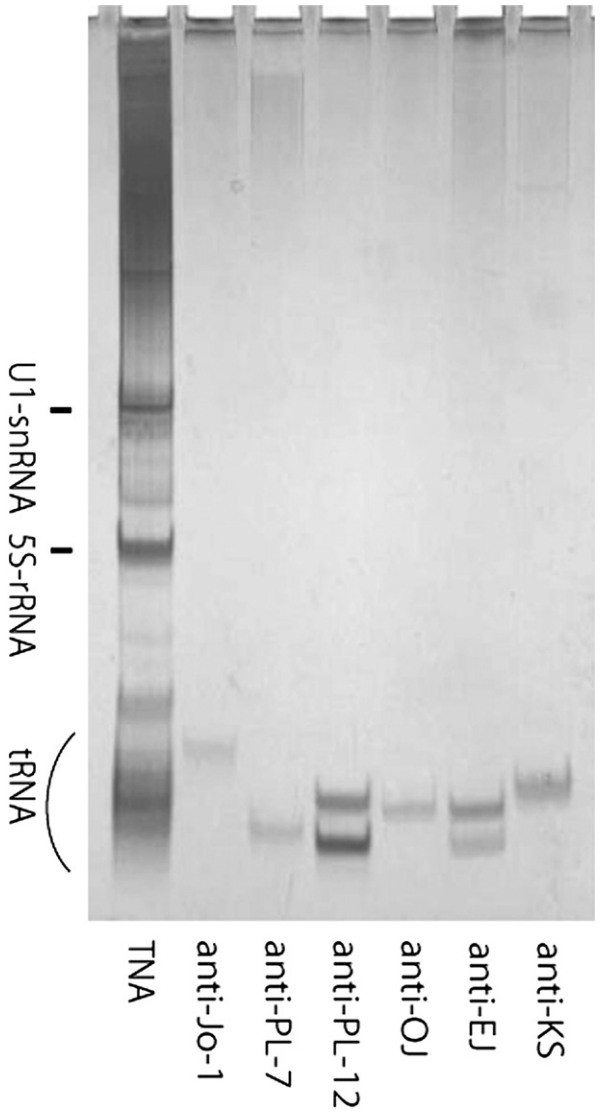

Figure 1 Immunoprecipitation of antibodies to antiaminoacyl-tRNA synthetases (anti-synthetase). The Figure demonstrates urea-polyacrylamide gel electrophoresis (PAGE) of immunoprecipitates, visualized with silver staining. Anti-synthetase antibodies are indicated in 6 lanes; antibodies to histidyl- (Jo-1), threonyl- (PL-7), alanyl- (PL-12), isoleucyl- (OJ), glycyl- (EJ), and asparaginyl-(KS) tRNA synthetases. Each antibody is distinguishable by the mobility and pattern of tRNA bands. TNA denotes total nucleic acids.

Results

Prevalence of anti-synthetase and clinical features

Anti-synthetase was found in 13 (6.6%) of 198 cases; anti-EJ was positive in 6 (3%), anti-PL-12 in 3 (1.5%), and anti-Jo-1, KS, OJ, and PL-7, one in each case (0.5%). No case exhibited reactivity to two or more antibodies. Median age at onset was younger in the anti-synthetase positive group than in the anti-synthetase negative group (55.0 vs. 67.4 years, respectively. $p < 0.001$, Table 1). Significant arthralgia or joint deformity, and cutaneous manifestations were

Table 1 Patient characteristics at initial presentation.

Measurements	Anti-synthetase positive ($n = 13$)		Anti-synthetase negative ($n = 185$)		p-value
Age at the onset (yr)	55.0	[44.5—60.8]	67.4	[60.6—73.2]	<0.001†
Female (n; (ratio %))	6	(46.2)	53	(28.7)	0.20
Smoking history (n)	9	(69.2)	129	(69.7)	0.78
Surgical biopsy for diagnosis (n)	8	(61.5)	36	(19.5)	<0.01†
Fever (>38 °C) (n)	2	(15.4)	10	(5.4)	0.18
Body weight loss (n)	1	(7.7)	9	(4.9)	0.50
Dyspnea (n)	12	(92.3)	154	(83.2)	0.70
Cough (n)	5	(38.5)	71	(38.4)	1.00
Other respiratory symptoms (n)[a]	1	(7.7)	9	(4.9)	0.50
Fine crackles (n)	12	(92.3)	167	(90.3)	1.00
Clubbed fingers (n)	1	(7.7)	34	(18.4)	0.47
Arthralgia or joint deformity (n)	2	(15.3)	4	(2.34)	< 0.01†
Raynaud's phenomenon (n)	1	(7.7)	4	(2.2)	0.29
Cutaneous symptoms (n)[b]	4	(30.8)	3	(1.6)	< 0.01†
Oxygen administration (n)	1	(7.7)	18	(9.7)	1.00
Treatment (n)	9	(69.2)	81	(43.8)	0.08
Corticosteroids	1	(7.7)	32	(17.2)	0.06
Corticosteroids plus immunosuppressants	8	(61.5)	49	(26.2)	
Immunosuppressants	0	(0.0)	1	(0.5)	
No medication	4	(30.8)	105	(56.1)	
Duration of the observation (months)	22.8	[17.0—35.1]	39.3	[17.0—65.0]	0.11
Survivor (n)	13	(100)	138	(74.6)	0.04*

Abbreviation: anti-synthetase = autoantibody to aminoacyl-tRNA synthetase. Numbers are expressed as median [25%—75% interquartile] or number of data (percent) Comparison was made by Fisher's exact test or chi-square test as appropriate; $p < 0.05$ *, $p < 0.01$ †. Treatment regimens included oral corticosteroids, immunosuppressants, and intravenous pulse methylpredonisolone therapy.

[a] Other respiratory symptoms include chest pain or hemoptysis.

[b] Cutaneous symptoms include Gottron's sign and heliotrope purpura.

frequent in anti-synthetase positive cases ($p < 0.01$). However, extrapulmonary features of ASS were absent in 6 anti-synthetase positive cases (46.2%). The number of cases in treatment tended to be higher in the anti-synthetase positive group. Survival rate at the end of the observation period was higher in the anti-synthetase positive group than in the anti-synthetase negative group ($p = 0.04$); however, survival curves did not reveal a significant difference between the two groups when analyzed with the log rank test ($p = 0.22$). All the NSIP survived during the study period regardless of anti-synthetase status; thus survival differences between anti-synthetase positive and negative NSIP were not analyzed. No case was diagnosed with PM/DM during the median observation period of 37.8 months (range 17.0—64.6) regardless of the results for anti-synthetase. Numbers of patients who met the criteria of UCTD in anti-synthetase positive and negative groups were 6 of 13 and 21 of 185, respectively. If patients with UCTD were excluded, the prevalence of anti-synthetase was 7 of 171 (4.1%).

Laboratory data, physiological measurements and BAL findings

In the anti-synthetase positive group, erythrocyte sedimentation rate (ESR) was significantly higher, and positive results for rheumatoid factor (RF) and anti—SS—A/Ro were more frequently seen when compared with the anti-synthetase negative group (Table 2). RF and anti-SSA/Ro were each concomitant with anti-synthetase in 4 cases (Table 2). There was no difference in the frequencies of other CTD-specific autoantibodies between ARS-Ab positive and negative groups (data not shown). The partial pressure of arterial oxygen (PaO_2) was significantly higher in the anti-synthetase positive group than in the anti-synthetase negative group ($p = 0.04$). The ratio of CD4+ to CD8+ T lymphocytes in the BAL fluids was predominantly <1 in the anti-synthetase positive group and >1 in the anti-synthetase negative group ($p < 0.01$), though the differences in differential cell counts were not statistically significant.

Pathological classification and findings

Biopsy specimens were obtained from 44 patients in this study; 8 were from anti-synthetase positive patients and 36 were from anti-synthetase negative patients. The numbers of patients who were diagnosed with NSIP and UIP were 11 and 30 respectively. The NSIP pattern was the most predominant (6/8, 75%) in the anti-synthetase positive group. (Table 3). Lymphoid follicles were observed in 7 of 8 cases (87.5%). Fig. 2 illustrates a typical case in the anti-synthetase positive group: observation of a pathological NSIP pattern with lymphoid follicles. Two cases in the anti-synthetase positive group were classified as pathological UIP pattern. While temporal heterogeneity, microscopic

Table 2 Laboratory test results at initial presentation.

Measurements		Anti-synthetase positive (n = 13)		Anti-synthetase negative (n = 185)		p-value
WBC	(/μl)	7300	[5850–12200]	7000	[5700–8600]	0.33
ESR	(mm/hr)	48.0	[33.0–67.0]	22.0	[12.0–41.5]	0.02*
CRP	(mg/dl)	0.9	[0.3–1.6]	0.2	[0.0–0.8]	0.03*
CPK[a]	(IU/L)	67	[39.3–113]	90	[60–121]	0.24
ALD	(IU/L)	5.6	[4.0–8.6]	4.8	[3.7–6.1]	0.27
LDH	(IU/L)	255	[219–342]	233	[198–275]	0.17
ANA(>x40)	(n)	7/12	(58.3%)	74/174	(42.5%)	0.37
RF(>x15)	(n)	4/8	(50.0%)	23/135	(17.0%)	0.04*
Anti–SS–A/Ro (>15.6)	(n)	4/11	(36.4%)	8/129	(6.2%)	<0.01†
Anti–SS–B/La (>10.0)	(n)	1/11	(9.1%)	3/130	(2.3%)	0.28
$PaCO_2$	(mmHg)	41.7	[36.8–44.6]	41.4	[38.4–44.4]	0.70
PaO_2	(mmHg)	85.4	[81.7–90.7]	78.1	[69.6–87.4]	0.04*
% VC	(%)	76.7	[63.5–100.7]	87.9	[71.2–101.5]	0.40
% FVC	(%)	74.9	[62.6–95.9]	87.6	[68.5–99.9]	0.32
% TLC	(%)	72.7	[62.1–90.8]	70.0	[57.3–82.3]	0.54
% RV	(%)	79.6	[71.9–92.5]	67.9	[51.0–87.6]	0.07
% Dlco	(%)	51.9	[35.8–76.0]	49.8	[37.0–62.6]	0.70
BALF findings[b]		(n = 9)		(n = 99)		
Total cell count	(/μl)	210	[123–293]	200	[100–400]	0.62
Neutrophil	(%)	4.0	[0.3–8.0]	6.0	[3.0–14.0]	0.15
Lymphocyte	(%)	20.0	[14.3–51.8]	15.5	[6.7–34.8]	0.14
Macrophage	(%)	61.5	[35.0–79.3]	67.0	[40.5–82.0]	0.75
Eosinophil	(%)	2.0	[1.1–7.3]	3.0	[0.5–5.0]	0.71
CD4/CD8 <1 (n)		7	(77.8)	30	(30.9)	<0.01†

Abbreviations: ALD = aldolase; ANA = antinuclear antibody; anti-synthetase = autoantibody to aminoacyl-tRNA synthetase; BALF = bronchioalveolar lavage fluid; CPK = creatine phosphokinase; CRP = C-reactive protein; Dlco = diffusion capacity; Dlco/VA = diffusion capacity per ESR = erythrocyte sedimentation rate; FVC = forced vital capacity; LDH = lactate dehydrogenase; PaCO2 = partial pressure of arterial carbon dioxide; PaO2 = partial pressure of arterial oxygen; RF = rheumatoid factor; RV = residual volume; TLC = total lung capacity; VA = alveolar ventilation; VC = vital capacity; WBC = white blood cell count. Numbers are expressed as median [25%–75% interquartile] or number of data (percent). Comparison was made by Fisher's exact test; $p < 0.05$ *, $p < 0.01$ †.

[a] Normal range of CPK is 35–141(IU/L).

[b] Number of patients: 9 in anti-synthetase positive and 99 in anti-synthetase negative patients.

honeycombing, and subpleural dense fibrosis suggested a UIP pattern (Fig. 3a), the observed moderate cellular infiltrate was different from a typical UIP (Fig. 3b). Histological UIP with lymphoid follicles were diagnosed in a total of 5 cases, and 2 were positive for the antibody. Therefore, among patients with NSIP or histological UIP with lymphoid follicles, the prevalence of anti-synthetase antibody was 8/16 (50%).

HRCT findings in the anti-synthetase positive group

HRCT findings in anti-synthetase positive cases are shown in Table 4. Interobserver variability (κ coefficient) ranged from 0.60 to 1.0. Abnormal findings were distributed predominantly in lower lung fields. Pleural irregularities and/or prominent interlobular septa, reticulation, ground glass opacity, and traction bronchiectasis or bronchiolectasis were observed in more than 80% of cases. Consolidation, subpleural lines, and irregular peribronchovascular thickening were observed predominantly in lower lung fields in 50% of cases. Honeycombing was not seen in any case. Even in cases with pathological diagnosis of UIP pattern, radiological findings did not follow a typical IPF/UIP pattern (Fig. 3c). Centrilobular nodular opacity was found in a substantial number of anti-synthetase positive cases (Fig. 4).

Discussion

We have demonstrated the prevalence of anti-synthetase among a substantial number of patients with IIPs to be 6.6% (13 of 198 cases). Measurements were carried out regardless of the presence or absence of extrapulmonary ASS-features. The anti-synthetase positive population was younger at onset and had an almost equal sex ratio. Nonspecific interstitial pneumonia (NSIP) pattern was a predominant pathological diagnosis in the anti-synthetase positive group and the most common HRCT findings were diffuse ground glass opacities in all lung fields and traction bronchiectasis in both lower lung fields. Even in cases which were pathologically diagnosed with UIP, radiographic features were not typical of IPF/UIP.

The patients with ILD and positive anti-synthetase are in the category of ASS without myositis. A previous study

Table 3 Summary of clinical manifestations, autoantibody profiles, pathological findings, and fulfillment of criteria of UCTD in patients with positive autoantibodies to aminoacyl-tRNA synthetases (anti-synthetase).

case no.	age and sex	anti-synthetase	CTD signs or symptoms	ANA	RF	other autoantibodies, inflammatory markers	pathological classification and notable findings	UCTD[b]
1	54 F[a]	EJ	monoarthritis	−	+	anti-CCP, SSA	fNSIP, lymphoid follicle	yes
2	43 M	EJ	−	x40	+	−	cNSIP, lymphoid follicle	−
3	61 M	EJ	−	x80	−	−	cNSIP	−
4	60 F	EJ	−	x40	−	−	UIP, cellular infiltrate, lymphoid follicle	−
5	41 M	EJ	finger tip hyperkeratosis	−	−	positive ESR, CRP		yes
6	62 M[a]	EJ	finger tip eczema, articular swelling	x40	−	anti-CCP		yes
7	43 M	PL12	fever, body weight loss, cutaneous manifestation	x40	+	anti-SSA, SSB		yes
8	52 M	PL12	−	x320	+	anti-SSA	fNSIP, lymphoid follicle	−
9	60 F	PL12	−	x40	−	−	UIP, cellular infiltrate, lymphoid follicle	−
10	54 M	Jo-1	finger tip desquamation	−	−	positive CRP		yes
11	58 F	PL7	sicca	−	−	−	fNSIP, lymphoid follicle	−
12	45 F	OJ	Raynaud's phenomenon	−	−	anti-SSA		yes
13	60 F	KS	−	−	−	−	cNSIP, lymphoid follicle	−

Abbreviation; ANA = antinuclear antibody; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; c/f NSIP = cellular/fibrosing nonspecific interstitial pneumonia; RF = rheumatoid factor; UCTD = undifferentiated connective tissue disease; UIP = usual interstitial pneumonia.

[a] These two cases were retrospectively categorized into early arthritis according to the new ACR/EULAR criteria published in 2010. These two cases were not classified into RA according to the 1987 ACR criteria. At the study entry, the new ACR/EULAR diagnostic criteria had not yet been published; therefore we did not exclude these two cases and listed them as anti-synthetase syndrome.

[b] The criteria of UCTD were by Kinder et al published in 2007.

showed that anti-synthetase was detected in a proportion of type 1 diabetes patients[21]; however another report showed its 100% specificity to ASS using the IPP method.[22] In this study, no study subject had concurrent type 1 diabetes. As in previous studies, the prevalence of pulmonary manifestation varied among the different antibodies.[4,23–26] Anti-Jo-1 is the most prevalent in patients whose muscular symptoms are obvious, and anti-PL7 and anti-EJ show the next highest prevalence.[3] However, anti-Jo-1 is less prevalent in patients whose muscular symptoms are absent or obscure.[7,27] Patients with positive anti-PL-12, anti-KS or anti-OJ tend to have ILD predominantly, rather than myositis.[27–30] In this study, anti-EJ was observed most frequently in as much as 2.7 percent of patients, while the prevalence of anti-Jo-1 was small. This result may reflect the difference in overall frequencies of each antibody among anti-synthetase positive patients and suggests that anti-Jo-1 antibody is relatively rare among anti-synthetase positive patients without myositis.

As to the clinical associations, younger age and increased CRP or ESR may be characteristic features of anti-synthetase positive cases. Among extrapulmonary features of ASS, arthralgia, joint deformity, or cutaneous symptoms were significantly observed in anti-synthetase positive cases. Extrapulmonary features of ASS including Raynaud's phenomenon, arthralgia, or muscular symptoms were absent in about half of anti-synthetase positive cases; this demonstrates the difficulty of deciding whether to test for screening of anti-synthetase based on presence of extrapulmonary symptoms of ASS alone. RF or anti-SSA is often concomitant in patients with anti-synthetase positive IPs, as reported in previous studies.[8,30] Furthermore, it is reported that coexistence of anti-SSA/Ro and anti-synthetase, particularly anti-Jo-1, is predictive of a more severe fibrosis score in HRCT and a reduced treatment response to immunosuppressants.[31,32] Therefore, measurement of anti-synthetase merits consideration in anti-SSA/Ro positive cases. Regarding the pulmonary function test results and BAL fluid findings, predicting the presence of anti-synthetase is difficult from these practical measurements. All the anti-synthetase positive cases survived during the study period; however the observation period was shorter in the anti-synthetase positive group. Because of the higher proportion of NSIP in the anti-synthetase positive group, their survival rate was expected to be better than in those without the antibody. However, this study did not show a conclusive difference in survival.

The impact of anti-synthetase on treatment response and prognosis, especially with common lung histopathology, needs to be defined in a future study involving a longer observation period.

The pathological diagnosis in ASS was predominantly NSIP. Even pathologically confirmed UIP showed some atypical features (Fig. 3b). Lymphoid follicles were remarkable findings in the anti-synthetase positive group (Table 3, Fig. 2c). Lymphoid follicle is commonly seen in lung biopsy specimens obtained from a case with CTD, particularly rheumatoid arthritis.[33] A recent study demonstrated that the germinal center score (i.e., number of lymphoid follicles per microscopic field) was the most distinguishing feature between CTD-related interstitial pneumonia (IP) and IPF/UIP.[34] The results of the present study

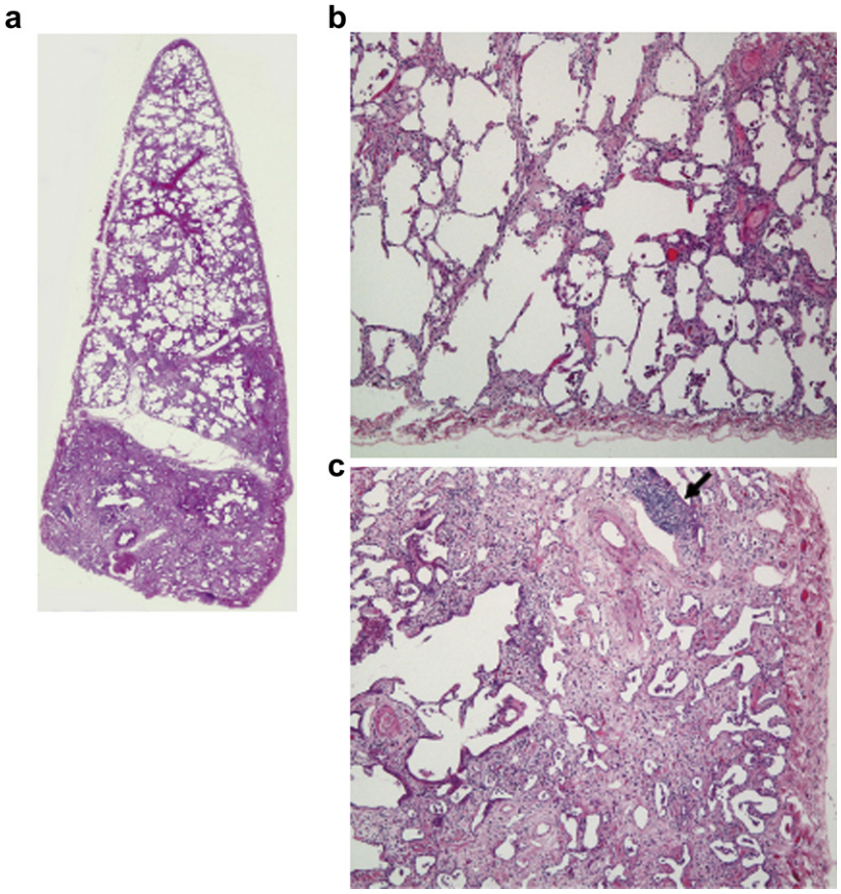

Figure 2 Pathological images in a patient with positive antibody to aminoacyl-tRNA synthetases (anti-synthetase). Pathological classification is nonspecific interstitial pneumonia (NSIP). 2-a) Low magnification view shows varying portions of inflammation and fibrosis distributed uniformly. 2-b) Lung architecture is generally preserved and there is mild to moderate interstitial fibrosis with cellular infiltrate. 2-c) In the more fibrotic area, the fibrosis is relatively loose. A lymphoid follicle and cellular infiltrate are also seen (arrow). No honeycombing is seen.

suggest that lymphoid follicle is characteristic also in anti-synthetase positive cases.

Chest CT revealed ground glass opacity distributed in all lung fields, traction bronchiectasis in lower lung fields and lack of honeycombing, all of which constitute essential HRCT findings characteristic of a non-UIP pattern. The HRCT findings of the current study were consistent with previous reports on interstitial pneumonia associated with PM/DM.[18] Our results were also in agreement with the American Thoracic Society report on NSIP, which found that the most consistent HRCT findings of NSIP are reticular opacities and traction bronchiectasis with lower lung zone predominance.[20] In idiopathic pulmonary fibrosis or idiopathic NSIP, centrilobular nodular opacity is not mentioned[1]; however in CTD-related lung disease such as rheumatoid lung disease, nodules are observed in pathological or radiological UIP or NSIP.[35] 30—40% of such nodules have been described as exhibiting centrilobular distribution. Centrilobular nodular opacity may be a finding characteristic of HRCT in anti-synthetase positive IIPs, but a rigid radio-pathological correlation was not established in this study.

Some cases with IPs exhibit specific autoantibodies or CTD signs or symptoms while they do not meet any specific criteria for CTD. A recent study reported that a large proportion of patients with idiopathic NSIP fulfilled the criteria for UCTD.[17] Indeed, 6 anti-synthetase positive and 21 anti-synthetase negative patients in our series also fulfilled the same criteria for UCTD, even without measurement of anti-synthetase. These cases were included in the study population because some patients with UCTD may be thought to overlap with ASS. Additionally, diagnostic criteria have yet to be fully established.[36,37] Some rheumatologists consider that cases with any disease-specific autoantibodies, including anti-synthetase, should be excluded from UCTD.[36]

Our results suggest that ASS-associated IPs have features in common with CTD-associated IPs but distinct from other IIPs, even if clinical CTD symptoms are subtle. Measurement of anti-synthetase may enable us to pick up such characteristics of otherwise "IIPs" cases, which may comprise a distinct entity. However, a larger cohort study should be conducted to address the best classification of such "IIPs" using certain clinical or serological CTD features.

All the anti-synthetase positive patients in this study presented with non-UIP pattern on HRCT and the prevalence of anti-synthetase antibody was as high as 50% among histological NSIP or UIP with lymphoid follicles. Thus, anti-

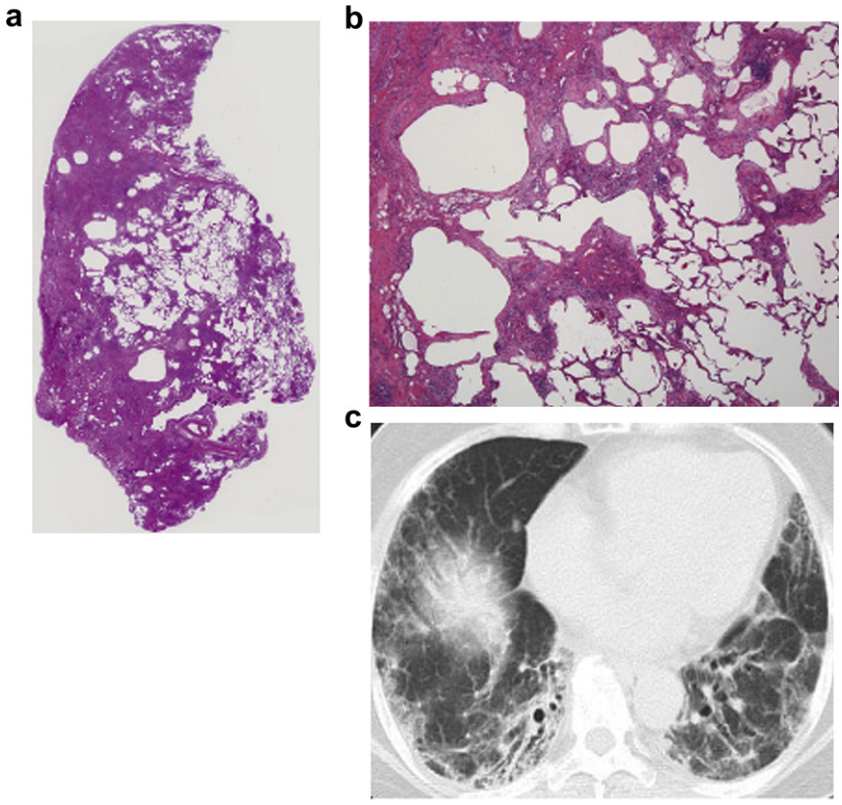

Figure 3 Pathological and radiographic images in a patient with pathological diagnosis of usual interstitial pneumonia (UIP). 3-a) Low magnification view shows subpleural dense fibrosis and normal alveolar architecture distributed in a patchy manner. 3-b) Fibrotic area shows temporal heterogeneity and relatively normal alveolar architecture is adjacent to the dense fibrosis. Fibroblastic foci are scattered. Mild to moderate cellular infiltrate is also seen. 3-c) Radiographic findings of the CT in lower lung fields show ground glass opacities, irregular peribronchovascular thickening and traction bronchiectasis, but do not reveal honeycombing.

synthetase antibodies should be screened for in patients with non-UIP pattern on HRCT, or histological NSIP or UIP with lymphoid follicles. Although all patients with radiologically typical UIP in our series were negative for anti-synthetase antibodies, our results were not conclusive regarding the significance of screening of these antibodies in such patients because of the relatively small sample size. A larger-scale study should be conducted to define the best candidates for anti-synthetase screening.

Previous reports have shown successful treatment of corticosteroid-resistant anti-synthetase positive ILD with immunomodulatory agents such as cyclosporin and

Table 4 Frequency of HRCT findings in patients with positive autoantibodies to aminoacyl-tRNA synthetases (anti-synthetase).

HRCT findings	κ coefficient[a]	Right[b]			Left[b]		
		upper	middle	lower	upper	middle	lower
Pleural irregularities and/or prominent interlobular septa	0.6	11 (85)	10 (77)	11 (85)	9 (69)	11 (85)	10 (77)
Reticulation	0.63	11 (85)	10 (77)	11 (85)	9 (69)	11 (85)	10 (77)
Ground glass opacity	0.67	8 (62)	12 (92)	13 (100)	8 (62)	12 (92)	13 (100)
Consolidation	0.67	0 (0)	2 (15)	5 (38)	0 (0)	1 (8)	7 (54)
Subpleural lines	0.66	3 (23)	6 (46)	8 (62)	3 (23)	6 (46)	9 (69)
Centrilobular nodular opacity	0.62	9 (69)	10 (77)	6 (46)	9 (69)	9 (69)	6 (46)
Irregular peribronchovascular thickening	0.92	0 (0)	1 (8)	6 (46)	0 (0)	1 (8)	7 (54)
Traction bronchiectasis and bronchiolectasis	0.73	1 (8)	6 (46)	11 (85)	0 (0)	6 (46)	10 (77)
Honeycombing	1.0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

[a] Kappa coefficient of the individual results of two readers.
[b] Data were expressed as number of patients (percent) judged through consensus to have the respective findings.

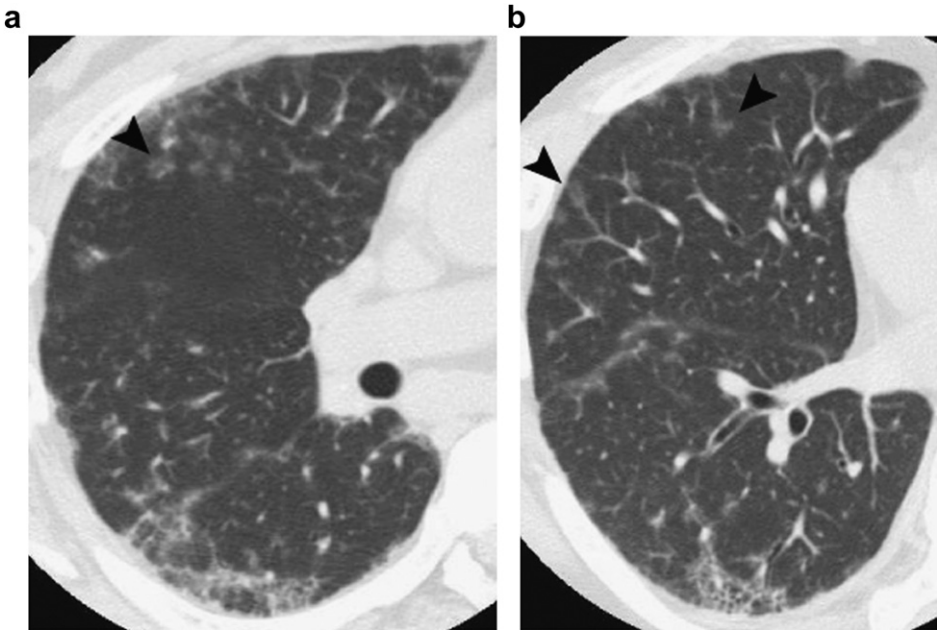

Figure 4 HRCT findings of centrilobular nodular opacity in an anti-synthetase positive case. Small-size nodules with ground glass densities are distributed in centrilobules (arrowhead) in the right middle lung (4-a) and in the right lower lung (4-b), respectively.

tacrolimus.[38] Considering the T lymphocyte involvement in the pathogenesis of ASS,[39] different treatment regimens may be applied for anti-synthetase positive ILD. In addition, all anti-synthetase positive cases have survived in the present study, suggesting that anti-synthetase positive IIPs may have a better prognosis. Although further examinations are necessary to confirm these hypotheses, we should stress the importance of measuring anti-synthetase in IIP for the identification of specific subgroups.

A limitation to our study was the retrospective study design. We could not enroll adequate numbers of patients who had obtained a pathological diagnosis of NSIP, to compare pathological and radiographic features in accordance with the anti-synthetase results. We recruited consecutive cases with IIPs regardless of the presence or absence of lung pathology in a search for the prevalence and characteristics of the anti-synthetase positive subpopulation. A large, prospective, longitudinal cohort of anti-synthetase positive cases would be required to characterize this clinical entity, incorporating treatment choice, response and survival.

In conclusion, an anti-synthetase positive subpopulation was not rare among IIPs. Anti-synthetase should be screened for in IIP patients, particularly in those with pathological NSIP or UIP with lymphoid follicles, even if no suggestive extrapulmonary manifestation exists.

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Conflict of interest

None of the authors have any financial or personal relationships with other people or organizations that could inappropriately influence (bias) the work reflected in this manuscript.

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